Cytotoxic Activity of Murine Platelets

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Platelets of intact mice and mice with tumors possess cytotoxic activity towards autologous and allogenic tumor strains. Platelet cytotoxicity significantly increases during tumor growth and decreases during the terminal stage. Murine platelets cause virtually no cytolysis of poorly differentiated (embryonal and strain unspecific) tumor cells.

Key Words: *murine platelet; cytotoxicity*

Normal human platelets and cells of cancer patients possess cytolytic activity towards continuous tumor cell lines [3,5,7]. The cytotoxicity of platelets of cancer patients varies depending on the stage of disease [1,2,4]. This activity was the highest during clinical stages II and III and significantly lower during stage IV in comparison with donors. However, published reports do not allow us to analyze the time course of killer activity of platelets during tumor progression. Therefore, we investigated cytotoxic activity of platelets of mice with transplanted tumors towards autologous and allogenic tumor target cells at various periods after implantation.

MATERIALS AND METHODS

C57Bl/6, CBA, BALB/c, and (C57Bl/6×CBA) F_1 mice of both sexes weighing 20-25 g aged 3-4 months from the Stolbovaya Breeding Center of the Russian Academy of Medical Sciences and the vivarium of the Oncology Research Center were used in the study.

The animals were subcutaneously injected 0.5 ml of tumor cell suspension in medium 199 with 40 μg/ml of gentamicin (750,000 cells per mouse). Tumor strains EL-4 and B-16 were donated by Dr. E. S. Revazova (EL-4, B-16 melanoma, Rag). Embryonal teratocarcinoma was induced at the Institute of Experimental Diagnosis and Therapy of Tumors by ectopic implantation of a 7-day mouse embryo to an adult mouse. After several passages, the tumor acquired capacity to grow in allogenic animals.

Tumors were palpated 5-7 days after implantation, and ascites were examined on days 8-10. Overall deaths were observed on days 18-21.

For further transplantations of tumors and their introduction in long-term cultures, tumor fragments without sites of necrosis were collected on days 10-15 after implantation and mechanically fragmented in medium 199 with gentamicin. Ascites was washed in medium 199, centrifuged at 200*g*, and the sediment was resuspended in culture medium. Cells were cultured in RPMI-1640 with 10% fetal bovine serum (FBS), 30 μg/ml of L-glutamine, 1% HEPES, and 0.4 mg/ml of gentamicin.

The effectors — murine platelets — were isolated from peripheral blood. In order to prevent rapid clotting, blood was collected from the retroorbital sinus with a sterile heparin-treated Pasteur pipette into a sterile glass tube with 0.5 ml of medium 199 with heparin (20 U/ml) or tube with citrate dextrose, rapidly mixed, and centrifuged for 5 min at 200*g* to sediment erythrocytes and leukocytes. A layer of plasma with platelets was collected. Platelets were sedimented by centrifugation (400*g*, 10 min), washed 3 times in medium 199 with heparin (the last washing without heparin), and resuspended in 1 ml RPMI-1640 with 5% FBS, 30 mg/ml of L-glutamine, 1%

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TABLE 1. Cytotoxicity of Platelets of Intact C57Bl/6, BALB/c, CBA, and (C57Bl/6×CBA) F_1 Mice Towards Tumor Cells Rag, EL-4, and B-16

Mouse strain	% of lysis of tumor strains		
	Rag	EL-4	B-16
C57Bl/6	27.75±4.7	37.51±3.4	26.75±5.4
BALB/c	47.1±2.8	42.85±3.6	54.1±4.6
CBA	—	25.5±3.3	—
(C57Bl/6×CBA) F_1	—	32.75±2.8	—

HEPES, and 40 μg/ml of gentamicin. The cells were counted, and suspension in medium 199 was transferred in 96-well flat-bottomed plates (100 μl per well) with target cells at 100:1 ratio. The total volume of the medium with target cells and platelets in the wells was no more than 200 μl.

Target cells were autologous tumor cells isolated immediately before experiment from ascites and cells from continuous cultures. In order to compare cytotoxic activities of platelets of intact mice and mice with implanted tumors, cells were isolated from the blood and tested with autologous and allogenic tumor cells.

Control group consisted of 26 mice, experimental of 51 mice. Platelet cytotoxicity was assessed in the MTT test after a 18-h incubation with target cells [6].

RESULTS

The cytotoxic activity of platelets of intact mice C57Bl/6, BALB/c, CBA, and (C57Bl/6×CBA) F_1 was assessed with autologous and allogenic tumor strains (isolated from ascites and cultured) EL-4, Rag, B-16, and T-20.

The efficacy of tumor cell lysis by intact mouse platelets varied from 16.3 to 59.1%.

Table 1 shows that platelets of BALB/c mice poorly susceptible to cancer (susceptible to milk factor but not to mammary cancer) more actively kill tumor strains EL-4, Rag, and B-16 than platelets of CBA and C57Bl/6 mice highly susceptible to cancer.

TABLE 2. Cytotoxicity of Platelets of C57Bl/6 Mice in a Panel of Continuous Tumor Cells

Mouse tumor cells	% of cytotoxicity
Rag — renal adenocarcinoma	27.75±1.7
B-16 — murine melanoma	26.75±1.46
EL-4 — T-cell lymphoma	37.51±3.42
T-20 — embryonal teratocarcinoma	13.0±1.12
Thym-B — thymoma	4.7±1.8

The cytolytic effect of platelets of intact C57Bl/6 mice was assessed with epithelial (Rag), embryonal (T-20), lymphoid (EL-4, Thym-B), and neurogenic (B-16) tumor strains with different histological and functional characteristics (Table 2).

The results of these experiments indicate that intact mouse platelets possess the highest cytotoxicity towards differentiated tumor strains Rag, B-16, and EL-4. Poorly differentiated embryonal strains T-20 and Thym-B which lost their strain specificity are virtually insensitive to the cytolytic action of platelets.

Experimental C57Bl/6 were injected tumor cell suspension subcutaneously (B-16) and intraperitoneally (T-20 and Thym-B). Cytotoxic effect of platelets of mice with tumors was assessed on days 7, 12, and 21 with autologous and allogenic target cells (Table 3).

The time course of platelet killer activity of C57Bl/6 mice with B-16 melanoma followed up in autologous B-16 and allogenic Rag target cells appears to reflect the status of antitumor immunity at different stages of tumor process. The maximum cytotoxicity of platelets was observed on day 12, which was apparently due to strained antitumor immunity; decreased tumor cell lysis by these effectors on day 21 can be regarded as a manifestation of immunosuppression caused by tumor expansion. On the whole, these results are in line with our previous findings in cancer patients. Platelet killing of tumor cells is increased during clinical stages II-III, whereas during stage IV platelet cytotoxicity is lower.

The cytotoxicity of platelets of C57Bl/6 mice towards autologous (B-16) and allogenic (Rag) tumor strains differed significantly only on day 12 after implantation. During this period, platelet activity towards both tested strains was the highest, but the cytotoxicity of effectors towards autologous cells was approximately two times higher than that towards allogenic strains. These results permit us to hypothesize that along with nonspecific platelet activity, platelets possess antigen-specific cytotoxicity.

The level of cytolytic activity does not depend on morphofunctional features of implanted tumors but is determined by histogenetic characteristics of tumor target cells (Table 4). Platelets of mice trans-

TABLE 3. Time Course of Cytotoxicity of Platelets of C57Bl/6 Mice with B-16 Melanoma Towards Autologous and Allogenic Tumor Cells

Tumor cell strain	% of target cell lysis in various periods after implantation, days		
	7	12	21
Rag	13.67±4.8	29.43±1.6	18.5±4.1
B-16	9.69±2.03	51.3±3.1	10.8±1.46

TABLE 4. Cytotoxicity of Platelets of C57Bl/6 Mice with Tumors B-16, T-20, and Thym-B Towards Tumor Cells B-16, T-20, and Thym-B

Mice with tumors	% of lysis of tumor target cells		
	B-16	T-20	Thym-B
B-16	24.6±5.35	6.5±1.21	6.3±1.42
T-20	30.5±2.6	12.5±0.96	0
Thym-B	20.5±2.74	7.75±1.5	11.75±1.24

planted different tumors (B-16, T-20, and Thym-B) caused comparable cytolysis of more differentiated melanoma B-16 cells, embryonal T-20 cells, and less mature Thym-B cells which lost their strain specificity.

Therefore, platelets of intact mice and animals with tumors possess cytotoxic activity towards autologous and allogenic tumor strains. The cytotoxicity appreciably increases in the course of tumor growth and decreases during the terminal stage. Mouse platelets caused virtually no cytolysis of poorly differentiated (embryonal and strain unspecific) tumor cells.

REFERENCES

1. S. N. Bykovskaya, N. G. Blokhina, D. L. Speranskii, and T. A. Kupriyanova, *Byull. Eksp. Biol. Med.*, **105**, No. 6, 708-709 (1988).
2. N. V. Malakhova, S. N. Bykovskaya, M. V. Kiselevskii, *et al.*, *Ibid.*, **119**, No. 3, 320-323 (1995).
3. G. N. Prizhivoit, *Vopr. Onkol.*, No. 3, 46 (1964).
4. S. N. Bykovskaya, A. V. Bolvacheva, M. V. Kiselevsky, and V. A. Khaylenko, *Biomed. Pharmacother.*, **45**, 279-288 (1991).
5. G. M. Ibele, N. E. Kay, and R. J. Johnson, *Blood*, **65**, 1252 (1985).
6. A. Loosdrecht, E. Nennie, G. Ossenkoppele, and R. Beelen, *J. Immunol. Methods*, **141**, 15-22 (1991).
7. K. Shimoda, S. Okamura, N. Harada, *et al.*, *J. Clin. Invest.*, **91**, 1310-1313 (1993).